UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

June 8, 2006

MEMORANDUM

Subject: Efficacy Review for NP 4.5 (D&F) Detergent/Disinfectant, EPA Reg. No. 1839-95; DP Barcode: D328118

From: Ibrahim Laniyan, Microbiologist
Product Science Branch
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Thru: Nancy Whyte, Acting Team Leader
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June 8, 2006

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

To: Velma Noble / Zenobia Jones
Regulatory Management Branch I
Antimicrobials Division (7510C)

Applicant: Stepan Company
22 West Frontage Road
Northfield, IL 60093

Formulation from the Label:

Active Ingredient(s)	% by wt.
Alkyl (60% C_{14}, 30% C_{16}, 5% C_{18}, 5% C_{12}) dimethyl benzyl Ammonium Chlorides	2.25 %
Alkyl (68% C_{12}, 32% C_{14}) dimethyl ethylbenzyl Ammonium Chlorides	2.25 %
Inert Ingredients	95.00 %
Total	100.00 %

I. BACKGROUND

The product, NP 4.5 (D&F) Detergent/Disinfectant (EPA Reg. No. 1839-95), is an EPA-approved disinfectant (bactericide, fungicide, virucide) and mildewstat for use on hard, non-porous surfaces in household, commercial, institutional, industrial, food processing, farm, animal care, and hospital or medical environments. The label claims that the product is effective in the presence of 5% serum. The applicant request to amend the registration of this product to include disinfectant claims against Avian Influenza A virus and SARS-associated coronavirus. Studies were conducted at MicroBioTest, Inc., located at 105 Carpenter Drive in Sterling, VA 20164; and ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated March 3, 2006), three studies (MRID Nos. 467795-01 through 467795-03), Statements of No Data Confidentiality Claims for the three studies, and the proposed label.

II. USE DIRECTIONS

The product is designed to be used for disinfecting hard, non-porous surfaces such as appliances, artificial turf surfaces, cabinets, cages, carts, coolers, counter tops, door knobs, floors, furniture, garbage cans, hospital beds and equipment, inflatable plastic structures, kennels, personal protective safety equipment, refrigerator/freezer exteriors, shelves and racks, shower curtains, showers, sinks, stovetops, telephones, toilets, tubs, urinals, walls, whirlpools, and windows. The proposed label also indicated that the product may be used on hard, non-porous surfaces including: glass, glazed ceramic, glazed porcelain, granite, marble, metal (e.g., chrome, stainless steel), plastic, and vinyl. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Add 2 ounces of the product per gallon of water (a 1:64 dilution). Apply the use solution with a mop, cloth, sponge, or sprayer. Wet all surfaces thoroughly. Allow surfaces to remain wet for 10 minutes. Remove excess liquid. For heavily soiled areas, a pre-cleaning step is required.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is

evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. These Agency standards are presented in DIS/TSS-2.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 467795-01 "Virucidal Efficacy Test, Avian Influenza virus" for NP 4.5 (D&F) Detergent/Disinfectant, by Lisa M. Lundberg. Study conducted at MicroBioTest, Inc. Study completion date – December 13, 2005. Laboratory Project Identification Number 123-231.

This study was conducted against Avian Influenza virus (Type A; Turkey/Wis/66; H9N2; obtained from SPAFAS), using embryonated chicken eggs (obtained from BE Eggs) as the host system. Two lots (Lot Nos. 3062-18 and 3062-29) of the product, NP 4.5 (D&F) Detergent/Disinfectant, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test, Avian Influenza virus," dated October 21, 2005 (copy provided). A use solution was prepared by adding 2 parts product to 126 parts sterile deionized water (a 1:64 dilution). The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried at ambient temperature. For each lot of product, separate dried virus films were treated with 2.0 ml of the use solution for 10 minutes at 21°C. Following exposure, 2.0 ml of newborn calf serum was added to each Petri dish. The plates were then scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was diluted serially in Earle's balanced salt solution. Embryonated chicken eggs (age not specified) were inoculated in quadruplicate via the allantoic route with 0.2 ml of selected dilutions. The eggs were incubated at 36±2°C for 2-4 days. Following the incubation period, the eggs were candled and then kept at 2±2°C overnight. Afterwards, the allantoic fluid was harvested and stored at less than -10°C until assay. The samples were analyzed for the presence of replicating virus using a hemagglutination assay. Controls included those for host viability/sterility, toxicity, toxicity-related viral interference, neutralizer effectiveness, and plate recovery count. The 50% embryo lethal dose/embryo infectious dose per ml (ELD/EID_{50}/ml) was determined using the method of Reed and Muench. The titer of the dried virus control was **6.0 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **4.5 $\log_{10}$** for both batches.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 467795-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A (H3N2) virus (Avian Reassortant)" for NP 4.5 (D&F) Detergent/Disinfectant, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – December 30, 2005. Project Number A03444.

This study was conducted against Avian Influenza A (H3N2) virus (Avian Reassortant) (ATCC VR-2072; Strain A/Washington/897/80 X A/Mallard/New York/6750/78), using Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories, Inc.; maintained in-house) as the host system. Two lots (Lot Nos. 3062-18 and 3062-29) of the product, NP 4.5 (D&F) Detergent/Disinfectant, were tested according to ATS Labs Protocol No. STE01102005.AFLU.2 (copy not provided). A use solution was prepared by adding 1 ml of the product to 63.0 ml of filter sterilized deionized water (a 1:64 dilution). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 20.0°C at 53% relative humidity for 20 minutes. For each lot of product, separate dried virus films were treated with 2.0 ml of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The titer of the dried virus control was **5.0 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **3.5 $\log_{10}$** for batch # 3062-18 and **4.5 $\log_{10}$** for batch # 3062-29.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

3. MRID 467795-03 "Virucidal Efficacy Test, SARS Associated Coronavirus" for NP 4.5 (D&F) Detergent/Disinfectant, by Lisa M. Lundberg. Study conducted at MicroBioTest, Inc. Study completion date – August 31, 2005. Laboratory Project Identification Number 123-217.

This study was conducted against Severe Acute Respiratory Syndrome (SARS)-associated coronavirus (obtained from ZeptoMetrix), using Vero E6 cells (obtained from ZeptoMetrix) as the host system. Two lots (Lot Nos. 3035-43-1 and 3035-43-2) of the product, NP 4.5 (D&F) Detergent/Disinfectant, were tested according to a MicroBioTest Protocol titled "Virucidal Efficacy Test, SARS-associated Coronavirus," dated December 14, 2004 (copy provided). A use solution was prepared by adding 1 ml of the product to 63.0 ml of sterile deionized water (a 1:64 dilution). The stock virus culture contained at least a 5% organic soil load. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried at room temperature for 30-60 minutes. For each lot of product, separate dried virus films were treated with 2.0 ml of the use solution for 10 minutes at 22°C. Following exposure, 2.0 ml of fetal bovine serum was added to each Petri dish. The plates were then scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were diluted serially in RPMI 1640 containing 10% fetal bovine serum. Vero E6 cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated at 35±2°C for 90-120 minutes for viral

adsorption. Post-adsorption, the cultures were aspirated, washed, refed, and incubated at 36±2°C for 7-10 days. Post-incubation, the cultures were scored for the presence or absence of unspecified cytopathic effects. Controls included those for cell viability/sterility, cytotoxicity, cytotoxicity-related viral interference, neutralizer effectiveness, and plate recovery count. The 50% cell culture infectious dose per ml ($CCID_{50}$/ml) was determined using the method of Reed and Muench. The titer of the dried virus control was **6.53 $\log_{10}$**. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was **4.03 $\log_{10}$** for both batches.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Cell viability control results were not reported in Table 3 of the laboratory report; however, the "Conclusions" section of the laboratory report stated that "[a]ll controls met the criteria for a valid test."

V. RESULTS

MRID #	Organism	Results			Plate Recovery Control
			Lot No. 3062-18	**Lot No. 3062-29**	
467795-01	Avian Influenza A virus	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.00}$ ELD/EID_{50}/ml
		ELD/EID_{50}/ml	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
467795-02	Avian Influenza A (H3N2) virus (Avian Reassortant)	10^{-1} dilution	Cytotoxicity	Complete inactivation	$10^{5.0}$ $TCID_{50}$/0.1 ml
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 ml	$\leq 10^{1.5}$	$\leq 10^{0.5}$	
		Log reduction	≥ 3.5 $\log_{10}$	≥ 4.5 $\log_{10}$	
			Lot No. 3035-43-1	**Lot No. 3035-43-2**	
467795-03	SARS-associated coronavirus	10^{-2} dilution	Cytotoxicity	Cytotoxicity	$\geq 10^{6.53}$ $CCID_{50}$/ml
		10^{-3} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$CCID_{50}$/ml	$10^{2.50}$	$10^{2.50}$	
		Log reduction	≥ 4.03 $\log_{10}$	≥ 4.03 $\log_{10}$	

VI. CONCLUSIONS

1. The submitted efficacy data **support** the use of the product, NP 4.5 (D&F) Detergent/ Disinfectant, as a disinfectant with virucidal activity against the following viruses on hard, non-porous surfaces in the presence of a 5% organic soil load for a contact time of 10 minutes at a 1:64 dilution:

Avian Influenza A virus MRID No. 467795-01
Avian Influenza A (H3N2) virus (Avian Reassortant) MRID No. 467795-02

Recoverable virus titers of at least 10^4 were achieved. In studies against Avian Influenza A (H3N2) virus (Avian Reassortant) conducted by ATS Labs, cytotoxicity was observed in the 10^{-1} dilution for one product lot. In studies against SARS-associated coronavirus, cytotoxicity was observed in the 10^{-2} dilutions. Complete inactivation (no growth) was observed in all other dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

In studies against Avian Influenza A virus conducted by MicroBioTest, Inc., complete inactivation was observed in all dilutions tested.

VII. RECOMMENDATIONS

1. **Please note**: The species name of the organism *Salmonella* ***choleraesuis*** has been changed by ATCC. The new designation of this organism is *Salmonella* ***enterica***. This change is effective immediately, and should be used for all subsequent references to this organism in the future.

2. The proposed label indicates that the product, NP 4.5 (D&F) Detergent/Disinfectant, is an effective disinfectant in the presence of 5% serum against SARS-associated coronavirus and Avian Influenza A for a contact time of 10 minutes at a dilution of 2 ounces per gallon of water (i.e., a 1:64 dilution). Data provided by the applicant **support** these claims.

3. Please make the following changes, as appropriate:

- Under the "Hatcheries" section on page 5 of the proposed label, add a statement such as "**For heavily soiled areas, a pre-cleaning step is required**."

- The current "Reusable Container Statement" on page 8 of the proposed label is not clear. Consider revising this statement to read: "A container, such as a spray bottle, can be reused once empty. First, prepare an appropriate use solution from concentrate. A dilution dispenser (or name specific equipment such as the Dynamix dispenser) can be used to prepare the use solution. Remove cap/spray nozzle from empty container. Fill empty container with newly prepared use solution. Replace cap/spray nozzle. Place correct use dilution label on the newly filled container."

- On page 9 of the proposed label, change "Keep **Out Reach** of Children" to read "Keep **Out of Reach** of Children."

- On page 4of the proposed label [left column; last paragraph], change "sanitize and **disinfectant**" to read "sanitize and **disinfect**."

- On pages 4 and 6 of the proposed label, change "**moonwalk**" to read "**moonwalks**" and change "obstacle coarse play" to read "obstacle course play."

- Under the "Cleansing of Body Surfaces . . ." section [see page 6 of the proposed label], change "**Bath** the entire body" to read "**Bathe** the entire body."

- Under the "Ultrasonic Bath Disinfectant Directions" [see pages 6-7 of the proposed label], change "**visible** dirty" to read, "**visibly** dirty."

I don't see where it applies